TOXICITY OF PERFLUOROSULFONATE (PFOS) IN CHICK EMBRYOS – LOAEL IS CLOSE TO CONCENTRATIONS IN PISCIVOROUS AVIAN TOP PREDATORS IN THE BALTIC SEA

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Introduction

A number of perfluorinated chemicals (PFCs), including perfluorooctane sulfonate acid (PFOS) and perfluorooctanoic acid (PFOA), are widespread in biota, including wild birds.[1,2] Scarce information on PFC toxicity to birds is available, and none is available for avian top predators. Dietary chronic laboratory studies have been conducted on PFOS effects in the mallard and the northern bobwhite quail, which yielded a LOAEL value for reproductive effects in female quail of 10 mg PFOS/kg and day.[3] In wild birds effects have been observed in great tits and blue tits collected near a fluorochemical plant in Belgium.[4] The hepatic PFOS concentration was positively correlated with serum alanine aminotransferase activity and liver weight and negatively with serum cholesterol and triglyceride levels. PFCs in other birds, such as in egg yolk samples of the top predator double-crested cormorant from Lake Winnipeg had PFOS concentrations of 0.130-0.320 mg/kg and ring-billed gull eggs from Lake Huron had egg yolk concentrations up to 0.150 mg PFOS/g.[1] Regarding levels in birds in the Nordic environment, PFOS was found in Glaucous gulls from Svalbard (0.0481-0.349 mg of PFOS/kg ww of plasma).[5] One of the highest measured PFOS concentrations in birds so far is in common Guillemot (*Uria aalge*) eggs from the Baltic Sea (1.023 mg/kg egg, ww).[6]

The numbers of identified perfluorinated chemicals (PFCs) in the environment are over 25 and probably increasing due to the versatile and widespread use, so the exposure situation for avian top predators has potential of becoming more and more complex. The most sensitive period of chemical exposure is often the embryonic period, and therefore qualitative and quantitative species-specific studies of effects of PFCs on bird embryos are warranted. There is a shortage of data on avian embryo-toxicity of PFCs and this information is essential in order to perform a risk assessment on PFC exposure in the guillemot and other avian top predators. Due to this, we have performed a study of the avian embryotoxicity of PFOS in chicken eggs. The chicken egg has proven to be a practical and sensitive model for studies of embryonal toxicity of halogenated hydrocarbons.[7] Since PFOS is found in high concentrations in egg yolk[6], a logic route of exposure is by injection into the egg yolk. For comparison of the effect of exposure route, air sac injections were also conducted.

Materials and Methods

PFOS was dissolved in water with an addition of 1% Tween-20 to increase the solubility of PFOS. One control group was exposed to 1% Tween-20 water solution (n=27) and another control group was exposed to pure water (n=25). One group was treated with 3 mg/kg egg (n=29), one group with 1 mg/kg egg (n=31) and one group was exposed to 0.3 mg/kg egg (n=30). Fertilized chicken eggs were obtained from a hatchery (Ova production AB, Morgongåva). They were stored below a temperature of 10° C until the start of incubation. The eggs were incubated in 37.5° C and at 60 % relative humidity. The eggs were automatically turned every 6 h. Before treatment, the eggs were candled and unfertilized or dead eggs were removed. At day 4 of incubation, the eggs were injected into the yolk sac via the air sac. The injection volume was 50 µl. A small hole was drilled in the shell of the blunt end of the egg and the injection was

made using a 1-ml syringe with a sterile injection needle. The hole was covered with paraffin and put back into incubation. The eggs were examined once every day of the incubation and mortality was recorded. At day 11 of incubation, the embryos were decapitated and examined for gross abnormalities. The embryos were weighed, as well as the livers and hearts. The liver weight was divided with the total embryonic weight to yield the liver somatic index (LSI). The same calculations were performed on the hearts (HSI). The LSI and HSI data were analyzed using the unpaired Student's t-test. The group treated with Tween-20 vehicle was used as control if nothing else is mentioned.

The air sac injections were done on day 7 of incubation, since earlier injections are difficult due to the small volume of the air sac. The treated groups were exposed to PFOS doses of 20 mg/kg egg (n=19) and 36 mg/kg egg (n=20) in 2% Tween-20 sterile water solution. The control groups were exposed to 2% Tween-20 water solution (n=19) or to pure water (n=19). A hole was drilled in the shell of the blunt end of the egg and the injection (300 µl) into the air-sac was made using a 1-ml syringe with a sterile injection needle. The hole was covered with paraffin and left with the site of injection facing upwards for 1 h before they were put back into incubation.

The survival rates were statistically analyzed using the Kaplan-Meier survival analysis with log rank test.

Results and Discussion

No gross abnormalities were noticed in any of the doses tested, but mortality was observed in all groups injected in the yolk. From figure 1 it can be seen that the different groups had different survival curves, with the highest PFOS exposed group (3 mg/kg) showing a rapid decline in number of surviving embryos already after day 6, i.e. after 2 days exposure. When comparing the survival curves of the Tween-20 control and the PFOS (3 mg/kg) group, the log rank test showed significant difference of the survival curves at day 11 with a p-value of 0.0230 (Fig. 1). This shows that PFOS had some effects that caused death early on in embryonic development.

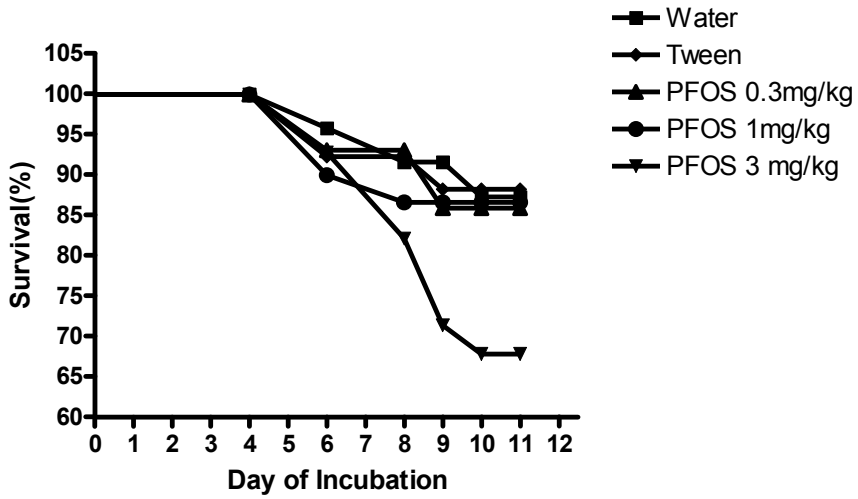

Figure 1. Survival rate in chicken embryos after egg yolk injections at day 4 of incubation (n=142).

The early mortality we have observed in chicken embryos contrasts to studies in rodents, where relatively unremarkable early embryotoxicity has been observed.[8] The mechanism of this early mortality in chicken

embryos is unknown, but it is known that PFOS can disrupt lipid metabolism. For instance, one major pathway affected by PFOS is peroxisomal fatty acid β- oxidation.[9] Thus the nutritional or energy status of the embryos may have been altered, resulting in reduced survival. Other possible mechanisms of PFOS embryotoxicity, such as interference with mitochondrial bioenergetics[10,11], disruption of cell–cell communication through gap junctions[12], interactions with fatty-acid binding proteins[13], hepatotoxicity (peroxisome proliferation) [14,15], and alterations of thyroid hormone economy[16] also warrant consideration. The total embryonic weight was on average slightly but significantly lower in the 3 mg/kg PFOS treated group compared to the Tween-20 control at D11 (data not shown).

The air-sac injections on day 7 of incubation also resulted in a rapid reduction in numbers of surviving embryos from 100% to 57% at day 11 but at a dose of 36 mg/kg egg, i.e. more than 10 times the dose used in the yolk-injection experiments (Fig. 2). The reason for the difference in toxicity is probably due to differences in uptake rate and bioavailability of PFOS from the air-sac compared to the yolk sac.

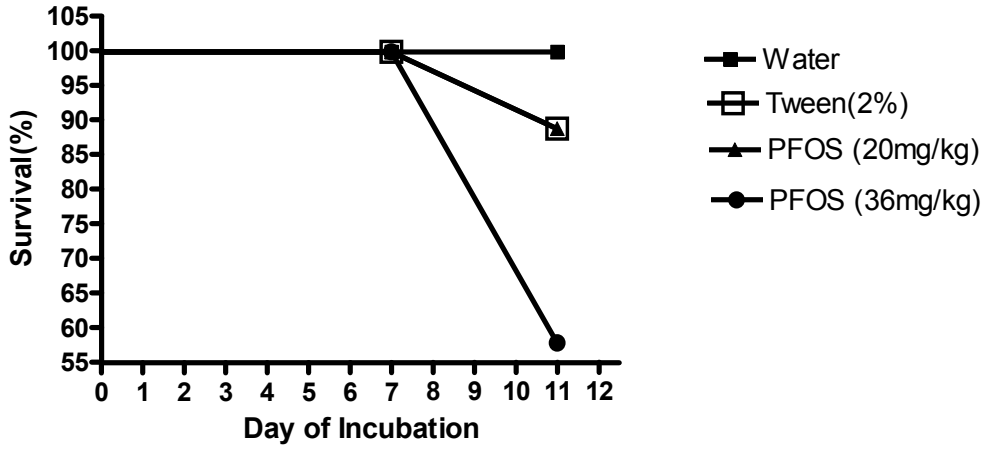

Figure 2. Survival rate in chicken embryos after air-sac injections at day 7 of incubation (n=77).

The LOAEL for mortality in chicken embryos after egg yolk injections was 3 mg/kg egg and NOAEL was 1 mg/kg egg, which is one of the lowest observed for avian species. Dietary chronic laboratory studies have been conducted on PFOS effects in the mallard and the northern bobwhite quail[3], which yielded a LOAEL value for reproductive effects in female quail of 10 mg PFOS/kg and day, equivalent to a concentration of 4.9 mg PFOS/kg, ww, liver. Regarding levels in birds in the Nordic environment, PFOS was found in Glaucous gulls from Svalbard (0.0481-0.349 mg of PFOS/kg ww of plasma).[5] One of the highest measured PFOS concentrations so far is in common Guillemot (*Uria aalge*) eggs from the Baltic Sea (1.023 mg/kg egg, ww)[6], which is similar to the NOAEL for mortality we have observed in chicken embryos (Figure 1). The fledging body mass of the common guillemot chicks at the St Karlsö breeding station during the period 1989-2001 showed a steady decline.[17] It is not easy to link this decline to PFOS exposure, but our experimental studies using in ovo chicken embryo exposure to PFOS show embryotoxic effects close to environmental concentrations in common Guillemot eggs from the Baltic Sea. The low margin of safety suggested in this study for the piscivorous top predator common Guillemot warrants further studies of avian species-specific sensitivities to PFOS and related PFC in order to enable accurate environmental risk assessment. Furthermore, it will be necessary to determine the critical effects of PFCs to be able to develop biomarkers to assess wild birds for responses to PFOS exposures. The early mortality syndrome warrants

further studies, possibly using ^{14}C-PFOS to study distribution in the egg and chicken embryos during the early post-injection period from incubation day 4 and onward to day 10. In addition, gene expression studies using a newly developed chicken gene array, containing 28,000 genes would increase chances of understanding possible mechanisms behind the early mortality.

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